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Original Research Article

Accumulation of Heavy Metal and Microbial Contamination of Smoked Catfish (Clarias gariepinus)

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Abstract: Recently, over 70% of fish are smoked as a means of preservation. Smoking is an ancient processing method that remains widely practiced in Nigeria today. This study investigates the levels of heavy metal accumulation and microbial loads in smoked catfish obtained from two different catfish farms to determine the safety of smoked catfish sold in Owo during the research period. Samples were collected from two farms (Farm 1 and Farm 2) in Owo, located in Owo Local Government Area, Ondo State, Nigeria. The identified microorganisms included Streptococcus spp., Staphylococcus aureus, Bacillus spp., Klebsiella spp., Pseudomonas aeruginosa, and Escherichia coli. The microbial counts for samples A and B were as follows: Streptococcus spp. (90.0 and 60.0), Staphylococcus aureus (160.0 and 170.0), Bacillus spp. (230.0 and 215.0), Klebsiella spp. (110.0 and 120.0), Pseudomonas aeruginosa (15.0 and 10.0), and Escherichia coli (2.0 and 1.0). For heavy metals, the concentrations were Cu (0.001 and 0.000), Cd (0.222 and 0.002), Cr (0.840 and 0.670), Mn (2.33 and 1.99), and Zn (132.020 and 127.001). The highest microbial count was observed in Bacillus spp. from Sample A (230.0) and Sample B (215.0), while the lowest was found in Escherichia coli from Sample B (1.0) and Sample A (2.0). Among the heavy metals, zinc was the most abundant in both samples, with Sample A (132.020) having a higher concentration than Sample B (127.001). Copper was the least abundant, being almost undetectable in Sample A (0.001) and entirely undetectable in Sample B (0.000). The study revealed variations in microbial and heavy metal contamination levels between catfish farms. It highlights the need for regulatory authorities to enforce moisture control measures and implement strategies to reduce human activities that may lead to bacterial growth and heavy metal contamination in smoked catfish products.

Keywords: Bacteria, microorganisms, contamination, heavy metals, smoked fish, catfish, and farms.

INTRODUCTION

Fish, belonging to the phylum Chordata and class Pisces, is predominantly aquatic (Saliu *et al*., 2013). It serves as an excellent source of essential minerals needed for proper body function (Elżbieta *et al*., 2015). Globally, fish is a significant source of protein, complemented by fats and oils beneficial for human nourishment. Catfish (Clarias gariepinus), widely consumed across socio-economic groups in Nigeria, serves as a vital source of animal protein, rivaling meat consumption. This freshwater species is highly regarded for its unique flavor, texture, and wide cultivation in ponds across the country. Fish also provides vital proteins, vitamins, and minerals, essential for both infant and adult diets (Abdullahi *et al*., 2001).

However, fish requires careful handling due to its susceptibility to spoilage in tropical temperatures, which accelerates bacterial, enzymatic, and oxidative processes (Eyo, 2004). In Nigeria, poor handling practices lead to significant post-harvest losses (30–50%). These losses can be minimized through appropriate processing and preservation methods, such as salting, drying, canning, and smoking (Bate & Bendall, 2010).

Smoking, a widely adopted method in Nigeria, involves applying heat to reduce water content and inhibit bacterial and enzymatic activity (Kumolu-Johnson *et al*., 2009). Despite its effectiveness, improper smoking methods and lack of hygiene often lead to high microbial loads and exposure to contaminants like dust and heavy metals. Studies (Abolagba & Iyeru, 1998) show that poorly smoked fish can harbor high microbial levels, and open-flame smoking may introduce cancer-promoting compounds into the body.

Heavy metals, characterized by high densities and atomic weights, include essential elements like iron, cobalt, and zinc, which are beneficial in small amounts but toxic in excess. Toxic heavy metals like cadmium, mercury, and lead are hazardous and often find their way into aquatic environments through activities like mining, agriculture, and industrial waste disposal. These pollutants are absorbed by catfish, posing potential health risks to consumers (Muradoglu *et al*., 2015).

This study aims to evaluate the levels of heavy metals and microbial loads in smoked catfish sourced from two farms in Owo, Ondo State, Nigeria.

MATERIALS AND METHODS

Sample Collection

Two catfish samples were obtained from two separate fish farms in Owo, Owo Local Government Area, Ondo State, Nigeria. The samples were transported in water to the Aquaculture Department at Rufus Giwa Polytechnic, where they were identified using standard procedures.

Sample Preparation

The catfish samples were washed thoroughly under running water and smoked without adding salt until fully dried. The smoked fish were separately crushed using a mortar and pestle, then milled into powder using a blender. The powdered samples were stored in airtight containers for laboratory analysis.

Materials

The materials used included HCl, H_2SO_4, a weighing balance, filter paper, heating mantle, crucible, thread, beaker, conical flask, distilled water, reagent bottles, chloroform, water bath, acetic acid, pipette, muffle furnace, and other necessary laboratory equipment. All chemicals utilized were of analytical grade.

DETERMINATION OF HEAVY METAL COMPOSITION

Ashing

Approximately 20 g of each powdered sample was placed in a crucible and ashed in a muffle furnace at 550 °C for 4 hours. The ashed samples were then removed and stored in a desiccator to prevent moisture absorption.

Digestion

The ashed samples were mixed with nitric acid (HNO_3) and hydrochloric acid (HCl) in a 1:3 ratio. The mixture was diluted to 100 ml with distilled water in a measuring cylinder, transferred into a beaker, filtered, and stored in sample bottles at room temperature for Atomic Absorption Spectrophotometer (AAS) analysis.

Atomic Absorption Spectrophotometer (AAS) Analysis

The concentrations of heavy metals, including cadmium, manganese, copper, zinc, and chromium, were determined in the samples using Atomic Absorption Spectrophotometer (AAS Model: 2000). The ash content and heavy metal analysis followed the AOAC (2010) method.

Microbial Analysis

Bacteriological Test

A standard plate count method was employed for the microbial analysis. Ten grams of each catfish sample were aseptically weighed and homogenized in 90 ml of sterile peptone water. Serial dilutions were prepared by mixing 1.0 ml of the suspension with 9.0 ml of sterile peptone water, producing a 10^{-1} dilution. Further dilutions were made up to 10^{-2}, and the diluted samples were spread onto nutrient agar plates to determine the total viable count.

Preparation of Agar Plates

Petri dishes were sterilized in a hot air oven at 160 °C for 1 hour. Nutrient agar was prepared by dissolving 0.6 g of agar in 100 ml of distilled water and sterilizing it in a microwave at 121 °C for 15 minutes. The agar was allowed to cool to 45 °C before use. All microbial analyses adhered to the procedures outlined by AOAC (2000).

RESULTS AND DISCUSSION

Results

Table 1: Showing the Samples and Location of Collection

Sample Local Name	Sample Botanical Name	Collection Point	Cold Room	State
Cat fish	Clarias gariepinus	Owo	Farm (1)	Ondo

Table 2: Showing the Samples and Location of Collection

Sample Local Name	Sample Botanical Name	Collection Point	Cold Room	State
Cat fish	Clarias gariepinus	Owo	Farm (2)	Ondo

Table 3: Showing Length (Cm and Inches) and Weigh (Kg and G) Of the Fish Samples

Fish Sample	L (Cm)	L (Inches)	Wt (G)	Wt (Kg)	Location
A (Cat fish)	30.50	12.007	1000	1.00	Farm 1
B (Cat fish)	30.70	12.087	1000	1.00	Farm 2

Table 4: Showing Results of Heavy Metal Composition of the fish Samples (mg/kg)

Fish Sample	Cu	Cd	Cr	Mn	Zn
A (Cat fish 1)	0.001	0.222	0.840	2.33	132.020
B (Cat fish 2)	0.000	0.002	0.670	1.99	127.001
WHO Limit (mg/kg) (1993)	4.00	0.05	0.050	5.00	99.4
FAO limit(mg/kg) (1999)	4.00	0.02	0.02	4.00	99.4

Discussion

The heavy metal contamination levels in the smoked catfish samples are summarized in Table 4 above. Zinc was the most abundant metal in both samples, with Sample A showing the highest concentration (132.020). Similarly, Sample B contained 127.001 of zinc. This aligns with findings by Ukulu *et al*., (2018), who reported a high zinc concentration (135.000) in catfish. The zinc levels in this study exceed the WHO (1993) and FAO (1999) recommended limit of 99.4 mg/kg. Zinc plays a crucial role in cell growth, division, and wound healing, but excessive intake can lead to adverse effects such as nausea, diarrhea, metallic taste, vomiting, kidney damage, and stomach issues.

Manganese (Mn) was also detected in both samples, with Sample A showing a higher concentration (2.33) compared to Sample B (1.99). These values are below the WHO (5.00 mg/kg) and FAO (4.00 mg/kg) limits for manganese in food. This indicates that the manganese levels in smoked catfish are insufficient to meet the body's requirements unless supplemented by other manganese-rich sources. Manganese is vital for iron metabolism, particularly in hemoglobin formation. These results can be compared to values reported by Igwemmar *et al*., (2013) for catfish (0.07 mg/kg), croaker (0.05 mg/kg), and tilapia (0.40 mg/kg), as well as those reported by Remi (2024) for roasted and fresh grass-cutter meat (0.111–0.114 mg/kg).

Chromium (Cr) levels in both samples were found to be high, with Sample A at 0.84 and Sample B at 0.67. These values exceed the limits recommended by WHO (0.05 mg/kg) and FAO (0.02 mg/kg). Kuton *et al*., (2021) reported lower chromium levels (0.11 and 0.054) in the liver and intestine of Malapterurus electricus. Excessive chromium intake can reduce insulin sensitivity, damage the liver and kidneys, and interfere with the efficacy of drugs like anti-inflammatories and paracetamol (Aslam & Yousafzai, 2017).

Cadmium concentrations were the lowest among the metals studied, with Sample B showing 0.002 and Sample A 0.222. Both values are below the standard limits of WHO (0.05 mg/kg) and FAO (0.02 mg/kg). Chima *et al*., (2017) reported the absence of cadmium in some fish samples, while Ukulu *et al*., (2018) found similar levels of 0.221 and 0.015 in catfish from Ajudabo, with undetectable levels in other samples. Cadmium, though present in low amounts here, is highly toxic and can cause kidney damage and bone demineralization at higher concentrations.

Copper (Cu) was detected only in Sample A (0.001), while it was undetectable in Sample B. Alinnor and Obiji (2010) reported significantly higher copper levels in fish, ranging from 1.247 to 8.00 ppm. The copper levels in this study can also be compared to the 0.199 and 0.212 ppm values for roasted and fresh grass-cutter meat, as reported by Remi (2024). The copper concentrations here were well below the WHO and FAO limit of 4.00 mg/kg. Copper is an essential trace element that supports enzymatic activity and overall metabolic functions.

Table 5: Results of microbial load of catfish samples (cfu/g)

Parameter	Sample A	Sample B
Streptococcus spp	90.0	60.0
Staphylococcus aureus	160.0	170.0
Bacillus subtilis	230.0	215.0
Klebsiella spp	110.0	120.0
Pseudomonas aeruginosa	15.0	10.0
Escherichia coli	2.0	1.0

The microbial loads (CFU/g) of the smoked catfish samples are detailed in Table 5. The bacterial isolates identified from the samples collected from the two catfish farms included Streptococcus spp, Staphylococcus aureus, Bacillus spp, Klebsiella spp, Pseudomonas aeruginosa, and Escherichia coli. The microbial loads in Sample A and Sample B were as follows: Streptococcus spp (90.0 and 60.0), Staphylococcus aureus (160.0 and 170.0), Bacillus spp (230.0 and 215.0), Klebsiella spp (110.0 and 120.0), Pseudomonas aeruginosa (15.0 and 10.0), and Escherichia coli (2.0 and 1.0).

Among the isolates, Bacillus spp had the highest load (230.0 and 215.0) in both samples, while Escherichia coli had the lowest (2.0 and 1.0), followed by Pseudomonas aeruginosa (15.0 and 10.0). These findings are consistent with those of Ariyo and Obire (2021), who reported low values of Escherichia coli and Pseudomonas aeruginosa in catfish samples. Similarly, Ayuba *et al.*, (2013) identified the presence of Escherichia coli and Staphylococcus aureus in smoked-dried sardine, corroborating the results of this study.

The detection of Staphylococcus aureus and Escherichia coli in the smoked catfish samples is consistent with Martin (1994), who noted that these microorganisms are commonly associated with smoked fish. Staphylococcus aureus, a bacterium frequently found on human skin and mucous membranes, is a leading cause of various human diseases. As Ayuba *et al*., (2013) also noted, Staphylococcus aureus can cause both superficial and systemic infections, including boils, pneumonia, and bacteremia. According to Salan *et al*., (2006) and Eyo (2001), Staphylococcus aureus is resistant to heat, drying, and radiation, and produces toxins that can lead to food poisoning, septic shock, and toxic shock syndrome.

Escherichia coli, often linked to improper fish handling and fecal contamination (Talaro, 2009), can cause diarrhea, kidney damage, and urinary tract infections (Salan *et al*., 2006; Eyo, 2001). The presence of Staphylococcus aureus and Escherichia coli in these samples poses risks of food poisoning and foodborne diseases, particularly if the contaminated fish is consumed.

The study also found Pseudomonas spp, an opportunistic pathogen associated with food spoilage and infections, in both smoked catfish samples. This contrasts with Olayemi *et al*., (2012), who reported the absence of Pseudomonas spp in catfish samples from Ibadan. Similar findings have been reported by Adegunloye and Sanusi (2019), Daramola *et al*., (2020), and Oku and Amakoromo (2013), who identified Bacillus spp and Pseudomonas spp in smoked fish samples from Tombia and Swale markets.

The presence of bacteria in the smoked catfish samples can be attributed to residual moisture (despite drying) and elevated temperatures that promote bacterial growth (Ibrahim *et al*., 2014). Additionally, Bacillus spp are known to produce endospores, allowing them to survive harsh environmental conditions.

Notably, significant differences were observed in the loads of Staphylococcus aureus and Klebsiella spp between the two samples, with values of 160.0 and 170.0 for Staphylococcus aureus and 110.0 and 120.0 for Klebsiella spp in Samples A and B, respectively. The highest load of Streptococcus spp was recorded in Sample A (90.0), compared to Sample B (60.0).

COCLUSION AND RECCOMENDATION

Conclusion

In summary, the levels of heavy metal and microbial contamination in smoked catfish products vary between different fish farms. This contamination is attributed to factors such as inadequate smoking processes, poor personal hygiene of handlers, unsanitary smoking environments, improper storage, inadequate packaging, and various human activities. These findings suggest that the two smoked catfish samples examined were contaminated not only during the handling process but also at the fish farm level due to the conditions mentioned.

Recommendation

Dehydration: Ensure complete dehydration of smoked catfish to inhibit bacterial growth caused by residual moisture.

Waste Management: Monitor and regulate human activities such as improper waste disposal, industrial discharges, and agricultural practices to reduce or eliminate heavy metal contamination in aquatic environments.

Collaborative Efforts: Regulatory bodies like the National Agency for Food and Drug Administration and Control (NAFDAC), the World Health Organization (WHO), and the Food and Agriculture Organization (FAO) should collaborate to establish and enforce moisture content standards for smoked catfish to minimize microbial growth.

Proper Drying: Catfish should be adequately dried before consumption, as heat can significantly reduce microbial contamination.

Public Awareness: Educate stakeholders, including fish farmers and consumers, on the importance of proper handling, smoking, and storage techniques to ensure food safety.

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